Exhibit R-2, RDT&E Budget Item Justification: PB 2015 Defense Health Program											Date: March 2014	
Appropriation/Budget Activity 0130: *Defense Health Program* / BA 2: *RDT&E*					R-1 Program Element (Number/Name) PE 0602787HP / *Medical Technology (AFRRI)*							
COST ($ in Millions)	**Prior Years**	**FY 2013**	**FY 2014**	**FY 2015 Base**	**FY 2015 OCO #**	**FY 2015 Total**	**FY 2016**	**FY 2017**	**FY 2018**	**FY 2019**	**Cost To Complete**	**Total Cost**
Total Program Element	3.558	1.160	1.182	1.117	-	1.117	1.222	1.242	1.331	1.153	Continuing	Continuing
241A: *Biodosimetry (USUHS)*	0.726	0.237	0.241	0.228	-	0.228	0.249	0.254	0.272	0.235	Continuing	Continuing
241B: *Internal Contamination (USUHS)*	0.376	0.124	0.125	0.119	-	0.119	0.131	0.133	0.143	0.124	Continuing	Continuing
241C: *Radiation Countermeasures (USUHS)*	2.456	0.799	0.816	0.770	-	0.770	0.842	0.855	0.916	0.794	Continuing	Continuing

The FY 2015 OCO Request will be submitted at a later date.

A. Mission Description and Budget Item Justification

For the Uniformed Services University of the Health Sciences (USUHS), Armed Forces Radiobiology Research Institute (AFRRI), this program supports developmental research to investigate new approaches that will lead to advancements in biomedical strategies for preventing, treating, assessing and predicting the health effects of human exposure to ionizing radiation. Program objectives focus on mitigating the health consequences from exposures to ionizing radiation that represent the highest probable threat to U.S. forces in current tactical, humanitarian and counterterrorism mission environments. New protective and therapeutic strategies will broaden the military commander's options for operating within nuclear or radiological environments by minimizing both short-and long-term risks of adverse health consequences. Advances in assessment, prognostication, and therapy in case of actual or suspected radiation exposures will enhance triage, treatment decisions and risk assessment in operational settings.

B. Program Change Summary ($ in Millions)	FY 2013	FY 2014	FY 2015 Base	FY 2015 OCO	FY 2015 Total
Previous President's Budget	1.193	1.216	1.241	-	1.241
Current President's Budget	1.160	1.182	1.117	-	1.117
Total Adjustments	-0.033	-0.034	-0.124	-	-0.124
• Congressional General Reductions	-0.001	-			
• Congressional Directed Reductions	-	-			
• Congressional Rescissions	-	-			
• Congressional Adds	-	-			
• Congressional Directed Transfers	-	-			
• Reprogrammings	-	-			
• SBIR/STTR Transfer	-0.032	-0.034			
• Reductions related to Departmental Efficiencies - Project 241A	-	-	-0.025	-	-0.025
• Reductions related to Departmental Efficiencies - Project 241B	-	-	-0.013	-	-0.013

Exhibit R-2, RDT&E Budget Item Justification: PB 2015 Defense Health Program					Date: March 2014
Appropriation/Budget Activity 0130: *Defense Health Program I* BA 2: *RDT&E*		R-1 Program Element (Number/Name) PE 0602787HP *I Medical Technology (AFRRI)*			
• Reductions related to Departmental Efficiencies - Project 241C	-	-	-0.086	-	-0.086

Change Summary Explanation

FY 2013: Realignment from Defense Health Program, Research, Development, Test and Evaluation (DHP RDT&E), PE 0602787-Medical Technology (AFRRI) (-$0.032 million) to DHP RDT&E PE 0605502-Small Business Innovation Research (SBIR) Program (+$0.032 million).

FY 2013: General Congressional Reductions to DHP PE, 0602115-Applied Biomedical Technology (-$0.001 million).

FY 2014: Realignment from Defense Health Program, Research, Development, Test and Evaluation (DHP RDT&E), PE 0602787-Medical Technology (AFRRI) (-$0.034 million) to DHP RDT&E PE 0605502-Small Business Innovation Research (SBIR) Program (+$0.034 million).

Reduces non-combat injury research funding in order to focus and continue the pace of progress in critical and high priority research areas for DHP RDT&E, PE 0602787-Medical Technology (AFRRI) (-$0.124 million).

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program			Date: March 2014
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787HP / *Medical Technology (AFRRI)*	Project (Number/Name) 241A / *Biodosimetry (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2013	FY 2014	FY 2015 Base	FY 2015 OCO #	FY 2015 Total	FY 2016	FY 2017	FY 2018	FY 2019	Cost To Complete	Total Cost
241A: *Biodosimetry (USUHS)*	0.726	0.237	0.241	0.228	-	0.228	0.249	0.254	0.272	0.235	Continuing	Continuing

The FY 2015 OCO Request will be submitted at a later date.

A. Mission Description and Budget Item Justification

Biodosimetry (USUHS): For the Uniformed Services University of the Health Sciences (USUHS), the mission and research objectives for biodosimetry are to assess radiation exposure by developing and providing biological and biophysical dosimetry capabilities for acute, protracted, and prior radiation exposures; to identify proper medical treatment of injuries to military personnel to sustain warfighting capabilities; and to reduce dose detection threshold and automate assays to permit a robust and rapid capability.

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
Title: Biodosimetry (USUHS)	0.237	0.241	0.228
FY 2013 Accomplishments: -Continued evaluation of a panel of radiation-responsive biomarkers in rodent animal model and extended the utility of the multiple parameter biomarker assay system for individual biodosimetry. -Evaluate a subset of combined hematological and plasma proteomic radiation-responsive biomarkers in the relevant dose (0-14 Gy) and time (6h – 7 d) range based on total body irradiation (60Co-gamma rays) model using two mouse strains with different radiation sensitivities. -Performed a preliminary statistical analysis for dose-discrimination of animal groups for different combinations of protein (Flt-3 Ligand, SAA, IL-6, G-CSF, TPO, and EPO) and hematological (lymphocytes, neutrophils, and ratio of neutrophils to lymphocytes) biomarkers. -Completed development of a mouse specific dose prediction algorithm for the ELISA-based technique and the multiplexed high-throughput platform biodosimetry device. -Demonstrated that an increase in dose estimation accuracy using 3 biomarkers compared with any individual biomarker. -Identified a subset panel of radiation biomarkers that permit radiation dose assessment in the presence or absence of wounding, which were successfully used on a high-throughput biodosimetry device evaluating blind test (radiation alone) samples. -Completed studies to evaluate the effect of thermal burns in combination with radiation on the panel of hematological and proteomic biomarkers; biomarker measurements are in progress. -Completed mouse radiation study to evaluate performance of a biomarker panel on a point-of-care biodosimetry device. Analysis of samples from dose-response calibration curve and blinded study completed. -Initiated development of a response category severity score system for acute radiation syndrome (ARS) in mice based on clinical signs and laboratory test to permit relating biomarker levels to radiation bioeffects severity.			

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
-Sustained efforts to provide necessary proof-of-concept dose-response data to transition combined proteomic and hematological concept for further development of diagnostic devices (i.e., hand-held, field deployable) and obtain necessary FDA approval. -Completed pilot study to evaluate the effects of stress and partial-body irradiation on hematology and proteomic radiation biomarkers. -Initiated pilot study to evaluate effects of dose rate on hematology and select proteomic radiation biomarkers. -Determined that low dose radiation resulted in hypomethylation of spleen DNA in irradiated mice in contrast to high dose radiation which resulted in both hypomethylation and hypermethylation of spleen DNA in irradiated mice. -Determined that overall epigenetic changes (multiple endpoints) were greater in low dose irradiated mice in contrast to high dose irradiated mice, which showed significantly more direct chromosomal damage. -Optimized protocol for preparation of interphase- cell chromosome aberrations; initiated studies to develop a novel approach to improve quality and yields of lymphocytes with condensed chromosomes for analysis of radiation-induced chromosome aberrations. -Sustained automation efforts related to establishing SOPs, sample tracking, image capture and processing, detection and high-throughput quantification of radiation-induced metaphase-spread dicentric-chromosome aberrations, and laboratory information management for rapid radiation dose assessment. -Initiated efforts to establish an in vitro intestinal epithelial cell organoid culture model to identify and validate gastrointestinal radiation biomarkers. -Submitted invention disclosure entitled on a promising new radiation biomarker to the Joint (USU & HJF) Office of Technology Transfer. -Filed joint (AFRRI/MSD) provisional patent application entitled: “Biodosimetry Panels and Methods” based in part on new biomarkers discovered in mouse studies.			
FY 2014 Plans: -Continue to evaluate protein biomarkers, hematological parameters, and clinical signs responses 1 day to 30 days after total-body irradiated and wounded mice at non-lethal, sub-lethal, and lethal radiation doses. -Complete the radiation/burn combined injury study to evaluate potential confounding effects of burn alone and when combined with radiation on radiation biomarker panel in a murine TBI (60Co gamma-rays) model. -Complete the cytokine (G-CSF) treatment study to investigate the modifying effects of cytokine treatment on radiation biomarker panel in a murine TBI (60Co gamma-rays) model. -Establish the dosimetry map for protracted (Low-Dose-Rate or LDR) 60Co irradiation for murine model; initiate comparison studies between LDR and prompt radiation on selected biomarkers in murine models. -Complete study evaluating effects of 5 different dose rates on hematology and select proteomic biomarkers. -Continue characterization of the mouse-specific response category severity scoring system for acute radiation syndrome (ARS) based on clinical signs, laboratory tests, and blood plasma proteomic biomarkers.			

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
-Investigate gender and age effects on evaluated panel of protein biomarkers in mouse model up to 7 days post irradiation. -Begin to evaluate the protein biomarkers, hematological parameters, and clinical signs ranging 1d – 7d in partial-body irradiated mice. -Continue to evaluate whether epigenetic markers can be used to discriminate low dose from high-dose radiation. -Determine if there is a chromosomal aberration difference between external radiation and internalized depleted uranium. -Evaluate whether the profile of chromosomal aberrations in human samples are able to discriminate uranium exposure from other toxic exposures. -Continue studies to establish an intestinal epithelial cell organoid model for use in biodosimetry studies. -Investigate impact of improving chromosome condensation on the ability to automate detection and counting of interphase chromosome aberrations. -Develop and integrate a spooler for automatic gene expression data inclusion from experiments and literature for indexing into the automated analysis system. -Evaluate applicability of new hardware, imaging tools, and suitability for use of mobile platforms and tablets in the automated chromosome aberration scoring system. -Contribute in the preparation of the summary report for FDA use on the diagnostic utility of combined hematological and proteomic approach in triage biodosimetry applications based on the combination of hematological and proteomic biomarkers results using murine model system. -Sustain efforts to provide necessary proof-of-concept dose-response data to transition combined proteomic and hematological concept for further development of diagnostic devices (i.e., hand-held, field deployable).			
FY 2015 Plans: -Sustain studies evaluating discovered new radiation-responsive biomarkers in animal models for early-phase and organ-specific bioindicators. -Continue to evaluate the protein biomarkers, hematological parameters, and clinical signs ranging 1 day to 30 days in total-body irradiated (and wounded) mice at non-lethal, sub-lethal, and lethal radiation doses. -Continue to evaluate the protein biomarkers, hematological parameters, and clinical signs ranging 1d – 30d in partial-body irradiated (and wounded) mice at non-lethal, sub-lethal, and lethal radiation doses. -Initiate studies to evaluate effects of even lower dose rates on hematology and select radiation biomarkers. -Complete characterization of the mouse-specific response category severity scoring system for acute radiation syndrome (ARS) based on clinical signs, laboratory tests, and proteomic biomarker profile. -Investigate dose-rate effects for low (photons) and high (mixed field neutrons and photons) linear energy transfer radiation quality for protein biomarkers in total-body irradiation animal models up to 7 days post irradiation. -Evaluate the combined utility of hematological and protein biomarkers for biodosimetry applications high (mixed field of neutrons and photons) LET total-body irradiations in total-body irradiation animal models.			

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
-Perform biodosimetry GLP studies in mouse total-body irradiation models to establish the algorithm for radiation dose assessment and dose-dependent discrimination of animal groups using combined hematological and proteomic profiles. -Sustain efforts to provide necessary proof-of-concept dose-response data to transition combined proteomic and hematological concept for further development of diagnostic devices (i.e., hand-held, field deployable) and obtain necessary FDA approval. -Determine whether epigenetic markers can discriminate between chronic low dose and repeated low dose exposures. -Determine whether epigenetic markers can discriminate between external radiation and internalized depleted uranium.			
Accomplishments/Planned Programs Subtotals	0.237	0.241	0.228

C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY13

-Expand the panel of radiation-responsive protein biomarkers using murine radiation models.
-Demonstrate the enhanced utility for the combination of multiple protein biomarkers and hematological parameters in murine (several mouse strains) radiation model for radiation dose and injury assessment as well as for survival prognosis.
-Complete study to identify radiation biomarkers useful as biomarkers for monitoring recovery using cytokine (G-CSF) treatment studies in the mouse TBI model.
-Establish optimal growth conditions for intestinal epithelial cell organoid culture model.
-Initiate assessment of partial-body radiation murine models over the protracted time period.
-Evaluate the radioresponse for three radiation biomarkers measured by commercial ELISA kits using the intestinal epithelial cell organoid culture model.
-Identify whether epigenetic markers can be used to discriminate low dose from high-dose radiation.
-Provide preliminary report on study investigating whether there is a chromosomal aberration difference between external radiation and internalized depleted uranium.
-Incorporate radiation bioinformatics (radioinformatics) capabilities, to include computational methods and data management tools to advance data collection, analysis, interpretation, and reporting of large data sets.

By FY14

-Identify radiation biomarkers that are dependent on exposure dose-rate and specific for various ARS subsyndromes.
-Demonstrate accurate radiological detection of radiation biomarker from biological samples into quartiles of doses 0-1 Gy, 1-3 Gy, 3-6 Gy, 6-10 Gy, and greater than 10 Gy.

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241A / *Biodosimetry (USUHS)*

-Provide preliminary report on mouse ARS category score system based on multiple biodosimetric endpoints (i.e., peripheral blood cell counts and radiation-responsive protein expression profile), taking into account animal body weight, and temperature in the mouse radiation model.
-Characterize partial-body radiation murine models over the protracted time period and compare results with prompt irradiation on selected biomarkers.
-Provide preliminary analysis of the enhanced utility of combined hematological and protein biomarkers for biodosimetry applications following photon and mixed field neutrons total-body irradiations in a total-body irradiation murine model.
-Identify subset of biomarkers useful for radiation dose assessment when confounded with thermal burns.
Complete report of select radiation biomarkers that are dependent upon dose-rate.
-Report on gender and age effects as well as the partial-body irradiation effects on the evaluated panel of protein biomarkers in mouse model.
-Submit samples from radiation-exposed intestinal epithelial cell organoid cultures for Liquid Chromatography-Tandem Mass Spectrometry analysis for novel radiation biomarker discovery.
-Measure specific methylation and histone changes using RTPCR in low dose and high dose bronchial cells.
-Measure chromosomal abberations in lymphocytes from gamma ray and depleted uranium exposed mice (spleen tissues).
-Measure intra-chromosomal aberrations using mBAND technology in human samples from individuals potentially exposed to toxic materials during deployment.
-Improve condensation of interphase chromatin into discrete chromosomes capable to be read through high-throughput image capture tools.
-Establish and incorporate Absorption Color Pigment (ACP) method for automated image extractors within CLASP.
-Provide report to validate specificity and sensitivity statistical models for the automated image system and analyses thereby testing CLASP efficiency.
-Evalute the applicability and efficiency of developed SOP's after inclusion of multi-parametric approaches within CLASP.

By FY15
-Characterize partial-body radiation murine models over the protracted time period and compare results with prompt irradiation on selected biomarkers.
-Provide necessary proof-of-concept dose-response data to transition combined proteomic and hematological concept for further development of diagnostic devices (i.e., hand-held, field deployable) and obtain the necessary FDA approval. Prepare preliminary report for FDA on combined utility of hematological and protein biomarkers for biodosimetry applications in two FDA-required animal models.
-Identify other radiation biomarkers that are dependent on exposure dose-rate.
-Validate dosimetric response of 3 biomarkers from IEC organoids exposed to 0-16 Gy gamma-ray radiation. Measure specific methylation and histone changes using RTPCR in low dose and high dose murine spleen samples.

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program			Date: March 2014
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787HP / *Medical Technology (AFRRI)*	Project (Number/Name) 241B / *Internal Contamination (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2013	FY 2014	FY 2015 Base	FY 2015 OCO #	FY 2015 Total	FY 2016	FY 2017	FY 2018	FY 2019	Cost To Complete	Total Cost
241B: *Internal Contamination (USUHS)*	0.376	0.124	0.125	0.119	-	0.119	0.131	0.133	0.143	0.124	Continuing	Continuing

The FY 2015 OCO Request will be submitted at a later date.

A. Mission Description and Budget Item Justification

Internal Contamination (USUHS): For the Uniformed Services University of the Health Sciences (USUHS), the mission and research objective for Internal Contamination is to determine whether the short-term and long-term radiological and toxicological risks of embedded metals warrant changes in the current combat and post-combat fragment removal policies for military personnel. Additionally, the biological effects of internalization of radioactive elements from Radiological Dispersal Devices (RDDs) and depleted uranium weapons, as well as therapeutic approaches to enhance the elimination of radionuclides from the body are being investigated.

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
Title: Internal Contamination (USUHS)	0.124	0.125	0.119
FY 2013 Accomplishments: -Initiated assessment of the ability of molecularly imprinted polymers to bind to potential internal contamination risks using an in vitro model system. -Determined that depleted uranium-induced leukemic cell transformation can be suppressed using a combinatorial approach targeting epigenetic alterations.			
FY 2014 Plans: -Determine the efficacy of molecularly imprinted polymers on reducing the body burden of internalized radionuclides using a rodent model system. -Validate combinatorial approach of depleted uranium-induced damage to cellular epigenetic machinery using an in vivo model.			
FY 2015 Plans: -Test novel leukemia countermeasures to determine if chemoprevention mechanism involves modification of chromatin regulation in depleted uranium-induced leukemia in vivo. -Design feasibility study to determine if non-radioactive metals can substitute as template molecules for high-specific activity radionuclides in the synthesis of molecularly imprinted polymers.			
Accomplishments/Planned Programs Subtotals	0.124	0.125	0.119

C. Other Program Funding Summary ($ in Millions)

N/A

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241B / *Internal Contamination (USUHS)*

C. Other Program Funding Summary ($ in Millions)

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY 2013

-Complete study on depleted uranium-induced alterations in DNA packaging.
-Evaluate ability of molecularly imprinted polymers to bind potential internal contamination risks.

By FY 2014
-Complete assessment of combinatorial approach for assessing depleted uranium-induced damage.
-Conclude evaluation of molecularly imprinted polymers as decorporation agents.

By FY 2015
-Initiate study to assess feasibility of using non-radioactive templates in the synthesis of molecularly imprinted polymers to radioactive metals.
-Complete in vivo study on the mechanism of depleted uranium-induced leukemia.

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program			Date: March 2014
Appropriation/Budget Activity 0130 / 2	R-1 Program Element (Number/Name) PE 0602787HP / *Medical Technology (AFRRI)*	Project (Number/Name) 241C / *Radiation Countermeasures (USUHS)*	

COST ($ in Millions)	Prior Years	FY 2013	FY 2014	FY 2015 Base	FY 2015 OCO #	FY 2015 Total	FY 2016	FY 2017	FY 2018	FY 2019	Cost To Complete	Total Cost
241C: *Radiation Countermeasures (USUHS)*	2.456	0.799	0.816	0.770	-	0.770	0.842	0.855	0.916	0.794	Continuing	Continuing

The FY 2015 OCO Request will be submitted at a later date.

A. Mission Description and Budget Item Justification

Radiation Countermeasures (USUHS): For the Uniformed Services University of the Health Sciences (USUHS), this program supports developmental, mission directed research to investigate new concepts and approaches that will lead to advancements in biomedical strategies for preventing, treating, assessing and predicting the health effects of human exposure to ionizing radiation as well as radiation combined with injuries(burns, wounds, hemorrhage). Research ranges from exploration of biological processes likely to form the basis of technological solutions, to initial feasibility studies of promising solutions. Program objectives focus on mitigating the health consequences from exposures to ionizing radiation, in the context of probable threats to U.S. forces in current tactical, humanitarian and counterterrorism mission environments. New protective and therapeutic strategies will broaden the military commander's options for operating within nuclear or radiological environments by minimizing both short-and long-term risks of adverse health consequences.

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
Title: Radiation Countermeasures (USUHS)	0.799	0.816	0.770
FY 2013 Accomplishments: - Demonstrated that gamma-tocotrienol (GT3) preferentially up-regulates expression of anti-apoptotic genes to promote intestinal cell survival. - Gamma-tocotrienol mobilizes hematopoietic, endothelial and stromal progenitor cells into peripheral blood. - Identified a panel of biologically important metabolomics biomarkers for gamma radiation injury in gastrointestinal system. - Investigated micro-RNA changes in mouse spleen and kidney after radiation and its modulation by gamma-tocotrienol - Demonstrated activation of Wnt signaling pathway after radiation in human hematopoietic progenitor CD34+ cells and in hematopoietic spleen tissue. - Initiated a pilot study with nano-GT3 to develop an oral formulation in mouse model. - Lipid peroxidation after ionizing irradiation led to apoptosis and autophagy. A book chapter was published (Kiang et al., In: Lipid Peroxidation, pp. 261-278, 2012). - Demonstrated significant radioprotective effects of 17-DMAG on bone marrow, mediated by increasing hematopoietic cells and mesenchymal stem cells. A manuscript was contingently accepted by Cell Biosci for publication. - Demonstrated radioprotective effects of 17-DMAG on ileum and lung, mediated by reducing epithelial apoptosis and crypt autophagy. A manuscript is in preparation. - Found that mesenchymal stromal cells exhibited adaptive redox response to stimulation with lipopolysaccharide inflammagen by remodeling tissue barriers. A paper was published (Gorbunov and Kiang, Oxidative Med Cell Longevity 2013:186795, 2013).			

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
- Found that mesenchymal stromal cells upregulated autophagy defense mechanisms in response to ionizing irradiation combined with bacterial challenge. A book chapter was published (Gorbunov et al., In: Protein interaction, pp. 23-44, 2012.). - Found that pegylated G-CSF displayed significant therapeutic efficacy after radiation injury by increasing survival, mitigating blood cell depletion and preventing splenomegaly. - Found that ciprofloxacin modulated cytokine/chemokine profile in serum, improved bone marrow repopulation, and limited apoptosis and autophagy in ileum after whole-body ionizing irradiation combined with skin-wound trauma. A paper was published (Fukumoto et al., PLoS One 8:e58389, 2013). - Found that ciprofloxacin displayed significant therapeutic efficacy after radiation combined injury by increasing erythrocyte generation and cellular ATP production. A manuscript is in preparation. - Established an animal model of radiation combined with hemorrhage, which showed that hemorrhage enhanced radiation damage to the bone formation and maintenance. - Hemorrhage increased radiation-induced mortality, bone marrow cell loss, and peripheral blood cell depletion. - Hemorrhage increased erythropoietin concentrations in blood and kidney, which was inversely correlated with bone marrow cell loss. - Hemorrhage enhanced the radiation-induced increases in IL-6, KC and G-CSF concentrations and decreases in IL-17a concentration in serum, suggesting the presence of inflammation. - Hemorrhage transiently enhanced radiation-induced C3 production but not C-reactive protein, suggesting the presence of a transient inflammation. - Serum procalcitonin concentration, measured by ELISA, distinguished induced exogenous bacterial infection within 24 h in sublethally irradiated mice and endogenous sepsis in morbid lethally irradiated mice as confirmed by bacterial culture. This procedure can be used to determine when to start early antimicrobial therapy and reduce mortality from sepsis. - A manuscript is in preparation to report that combination therapy with a nonspecific immunomodulator, synthetic trehalose dicorynomycolate and monophosphoryl lipid A (STDCM-MPL), and the antimicrobial agents, levofloxacin and amoxicillin, eliminates polymicrobial sepsis and extends survival in combined injured mice as well as in mice only lethally irradiated. - A nonspecific immunomodulator, synthetic trehalose dicorynomycolate and monophosphoryl lipid A (STDCM-MPL), increased serum concentrations of several cytokines and chemokines during the seven days after lethal irradiation or combined injury in mice. Mouse serum samples were analyzed and evaluated statistically for responses of interleukin-1α (IL-1α), IL-1β, IL-6, IL-10, granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon γ (IFNγ), keratinocyte-derived chemokine (KC), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1 (MIP-1-alpha and MIP-1-beta), and tumor necrosis factor-α (TNF-α). A manuscript is in preparation to report the findings. - Determined that 17-DMAG exacerbates radiation-induced reductions in trabecular bone microarchitecture, strength, and cellular activity. (Manuscript in preparation) - Determined that radiation exposure, as low as 1 Gy, negatively alters biomarkers of bone metabolism and results in significant reductions in trabecular bone			

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
- 8 Gy dose of ionizing radiation further exacerbates negative effects of radiation injury on bone - Determined that non-lethal radiation combined with skin wound trauma enhances the effectiveness of ionizing radiation to induce skeletal tissue damage and increase fracture risk (reduce bone strength) - Combined injury (8 Gy) induces bone loss that occurs as early as 7 days post-exposure and continues for at least 120 days (Manuscript in preparation) - Cancellous, not cortical, bone is more susceptible to combined injury-associated reductions in bone - Although a dose of radiation as low as 1 Gy is severely detrimental to bone, there appears to be a dose-dependent effect of radiation injury and combined injury on bone (8 Gy > 1 Gy) - Mice exposed to combined injury (8 Gy) experienced inhibited body mass accrual and did not recover this loss until 21 days after injury. - Determined that multiple administrations of recombinant mouse IL-10 (rmIL-10) attenuated wounding- and combined injury-associated reductions in red and white blood cells, neutrophils, lymphocytes, and leukocytes (Day 30). - rmIL-10 mitigated RI-induced reductions in lymphocytes and nearly doubled neutrophil levels in sham mice (Days 7 and 30). - rmIL-10 prevented reductions in spleen and liver mass after RI (Day 30). - Determined that rmIL-10 was unable to prevent early reductions in body mass after radiation and combined injury - Demonstrated accelerated wound healing with rmIL-10. Combined injury mice treated with rmIL-10 significantly reduced time to wound closure (16.4 ± 0.6 days) compared to vehicle treated CI mice (19.6 ± 1.8 days). - Determined that in the bone marrow microenvironment, reactive oxygen species (ROS) are critical to development of radiation leukemia, providing evidence of a new target for radiation-leukemia prevention. - Determined that epigenetic mechanisms and gene silencing controls are dysregulated during radiation-induced leukemia and may be a target for new therapies. - Determined that chromosomal instability (genetic change) is associated with radiation-induced leukemia and that non-targeted radiation damage is involved. - Determined that Phenylbutyrate treatment can prevent neoplastic transformation and genomic instability of bronchial airway cells at regardless of the type/quality of radiation. - Investigated feasibility of studies in irradiated minipigs evaluating changes in gene and protein expression signatures. Tocopherol succinate (TS)-mobilized progenitors significantly protected mice when administered as late as 48 h post-irradiation with 11.5 Gy and also mitigated radiation injury in gut. - TS mobilized progenitors inhibited apoptosis, and stimulated mitosis (cell proliferation), and inhibited gut bacterial translocation in high dose-irradiated mice in gut tissue. - TS mobilized progenitors also inhibited translocation of gut bacteria to various organs in mice irradiated with high dose of radiation causing GI injury. -Studied transcriptomes in mice tissue administered with TS and G-CSF antibody to understand the mechanism of action of TS and G-CSF.			

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
Appropriation/Budget Activity 0130 / 2	**R-1 Program Element (Number/Name)** PE 0602787HP / *Medical Technology (AFRRI)*	**Project (Number/Name)** 241C / *Radiation Countermeasures (USUHS)*

B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
- Study effect of TS mobilized progenitors in combined injury model (radiation exposure and wounding). - By analyzing transcriptomic signatures after TS stimulation and modulation of colony-stimulating factor production using functional genomics, determined the mechanism and necessary molecular components by which TS mediates colony-stimulating factor production and provide radioprotection. - Screened 10 new agents for radiation countermeasure efficacy. - Determined the efficacy of delta-tocotrienol in reducing radiation-induced clastogenicity. - Demonstrated that DT3 as an anti-apoptotic agent inhibited pro-inflammatory cytokine production and PTK6 expression in mouse intestinal tissue after exposure to γ-radiation and protected mice from lethal-dose radiation-induced acute gastrointestinal syndrome. - Demonstrated that REDDI1 (regulated in development and DNA damage responses), a novel survival factor, protects osteoblast cells from gamma radiation-induced premature senescence. - Demonstrated that human hematopoietic stem and progenitor cells and their niche cells have different miRNA expression patterns after irradiation and miR-30c plays a key role in radiation-induced cell damage which might be through regulation of REDD1 expression.			
FY 2014 Plans: - Evaluate the radioprotective and mitigative/therapeutic effects of nano-GT3 in mouse model - Determine acute and late effects of radiation-induced bone damage and prevention by gamma-tocotrienol after whole body radiation - Analyze global protein profiling after radiation in mouse spleen and kidney with varying doses and times after radiation. - Evaluate radiation-induced micro-RNA changes in mouse jejunum after gamma-tocotrienol treatment. - Evaluate the efficacy of a combined pharmaceutical regimen against radiation combined injury (irradiation followed immediately by skin wound trauma). - Determine effectiveness of combined therapy of G-CSF and ALXN4100TPO, a thrombopoietin receptor agonist, to prevent, mitigate, or inhibit the long-term deleterious responses to radiation combined injury. - Evaluate the micro-RNA profile in mouse serum after radiation alone and combination with wound trauma. - Evaluate the efficacy of IL-10 as a countermeasure to radiation and combined injury-associated effects on bone microarchitecture, strength, tissue-level cellular mechanisms, biomarkers of bone metabolism and immune effects. - Explore the role of the immune system in bone's response to radiation and combined injury (i.e. osteoimmunology). - Investigate the molecular mechanisms involved in radiation, wounding, hemorrhage, and/or combined injury. - Explore the role that sclerostin, an inhibitor of osteoblastogenesis, has on radiation and/or combined injury-associated reductions in bone mass and its effects on Wnt/β-catenin signaling. - Determine whether protection of bone marrow environment epigenetic changes following radiation can prevent radiation leukemia.			

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
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B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
- Continue study of the mitigation of radiation injury using apoptotic pathway markers in mice receiving TS-mobilized progenitors. - Perform genome-wide transcriptomic and proteomic profiling to elucidate coordinate pathway activation markers associated with tocopherol-mediated bioactivity. - Perform RNA-sequence profiling of small RNA, as well as mRNA transcriptomes, antibody microarray and 2D gel electrophoresis profiling of low and high abundance proteomes with samples obtained after TS treatment. - Small molecule inhibitors for candidate signaling pathways associated with TS activity will be utilized to determine their requirements for CSF family member production, most notably, G-CSF production. - Screen several human primary organ-specific cell types (epithelial, fibroblast, endothelial, etc.) for CSF transcript up-regulation in response to alpha-tocopherol. - Determine radioprotection (drug administered before irradiation) with 10 new compounds. - Elucidate radioprotection by BB-001 and ODSH. - Determine the efficacy of filgrastim (administered after irradiation) and ALXN4100TPO (administered prior to radiation) on radiation lethality and how the combination influences hematopoietic end points as measured by circulating blood elements. - Test efficacy of ALXN4100TPO in different mouse strains. - Evaluate microRNAs and inflammatory factors as radiation biomarkers. - Evaluate the radioprotective and mitigative/therapeutic effects of tilorone hydrochloride in in vivo animal model. - Study the role of inflammatory pathways in ionizing radiation-induced bone marrow failure. - Establish 3 dimensional coculture in vitro model to evaluate the effects of bone marrow endothelial cells (BMEC) on hematopoietic stem and progenitor cells (HSPC) in a 3D environment - Initiate ex vivo culture of murine BMEC for in vivo studies - Test hypothesis that EC improve animal survival after gamma irradiation - Test functional roles of EC in hematopoietic support after irradiation - Test hypothesis that Ang/Tie2 pathway is involved in animal survival after irradiation - Test functional roles of Ang/Tie2 pathway in hematopoietic support after irradiation - Initiate analysis of gene array data from irradiated human marrow endothelial cells and hematopoietic progenitor cells			
FY 2015 Plans: - Evaluate RANKL-mediated signaling pathways in skeletal tissues after radiation and their modulation by gamma-tocotrienol. - Examine radiation-induced neuronal damage and mitigation by gamma-tocotrienol using cell culture and mouse brain. - Evaluate the role of nrf2 pathway after radiation in microglial cells and its modulation by gamma-tocotrienol - Evaluate intracellular signaling pathways in mechanisms of efficacy of GT3 in different mouse tissues after radiation. - Determine the role of hedgehog signaling in hematopoietic recovery following sub-lethal dose of radiation (in vitro and in vivo study). - Determine the role of HIF-1a and HIF-2a in the regulation of erythropoiesis after radiation, and effect of gamma-tocotrienol.			

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
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B. Accomplishments/Planned Programs ($ in Millions)	FY 2013	FY 2014	FY 2015
- Continue to evaluate intracellular signaling pathways and cytokine profiles in mechanisms of efficacy of G-CSF and ALXN4100TPO in combined injured mice. - Continue to evaluate micro-RNA profiles in mouse serum after both radiation alone and combination with wound trauma with treatment with countermeasures. - Determine the potential efficacy of a sclerostin antibody, which inhibits radiation-induced reductions in bone formation. - Continue to explore the role of the immune system in bone's response to radiation and combined injury. - Determine whether Phenylbutyrate-induced suppression of neoplastic transformation of bronchial tissue is radiation dose-dependent (low versus high) and whether epigenetic or genetic processes are predominant. - Study transcriptomics in various subsets of TS-mobilized progenitors.			
Accomplishments/Planned Programs Subtotals	0.799	0.816	0.770

C. Other Program Funding Summary ($ in Millions)

N/A

Remarks

D. Acquisition Strategy

N/A

E. Performance Metrics

By FY 2014

- Complete evaluation of the therapeutic effects of G-CSF and ALXN4100TPO on survival after radiation combined injury.
- Complete evaluation of the micro-RNA profile in mouse serum after radiation alone and combination with wound trauma.
- Complete evaluation of IL-10 as a countermeasure to radiation combined injury-induced bone loss and effects on immune system.
- Complete evaluation of molecular mechanisms involved in radiation, wounding, hemorrhage, and/or combined injury.
- Complete determination of the role that sclerostin has on radiation and/or combined injury-associated reductions in bone mass and its effects on Wnt/β-catenin signaling in bone.
- Measure methylation and histone changes in radiation-leukemogenic mice
- Unfold part of underlying mechanisms of therapeutic effects of G-CSF, TS-mobilized progenitors, and ALXN4100TPO after radiation combined injury.
- Complete studies on CDX-301 mechanism(s) of action.
- Complete DRF studies with filgrastim using our optimized schedule.
- Repeat strain survival studies to determine LD50 in four mouse strains.
- Establish supportive care in Rhesus macaque model to include antibiotic treatment, blood transfusions and thereby establish LD-50 in primates.

Exhibit R-2A, RDT&E Project Justification: PB 2015 Defense Health Program		Date: March 2014
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By FY 2015
- Elucidate the fundamental underlying mechanisms of therapeutic effects of G-CSF and ALXN4100TPO after radiation combined injury.
- Begin determining the potential efficacy of a sclerostin antibody to inhibit combined injury-induced bone loss.
- Evaluate effect of chronic or repeated low dose radiation on neoplastic transformation of bronchial tissue.
- Initiate investigations into mechanisms of mitigation/protection by BB-001. Determine optimum dose and time schedules, followed by DRF studies.